# Localization and Characterization of Photosystem II in Grana and Stroma Lamellae[1]

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## ABSTRACT

Attempts have been made to identify intramembranous particles observed in freeze-fracture electron microscopy as specific functional components of the membrane. The intramembranous particles of the exoplasmic fracture (EF) face of freeze-fractured pea (*Pisum sativum*) chloroplast lamellae are nonuniformly distributed along the membrane. Approximately 20% of the particles are in unpaired membrane regions whereas 80% are localized in regions of stacked lamellae (grana partitions). The EF particles within the grana regions of the chloroplast membrane are of a larger average size than those in stroma lamellae.

Photosystem II activity of isolated stroma lamellae is about 20 to 25% of that of grana-enriched membrane fragments when measured at high light intensities. The photosystem II activity of stroma lamellae requires higher light intensities for attainment of maximal rates than does that of grana membranes. Lactoperoxidase-catalyzed iodination of stacked chloroplast lamellae was used to demonstrate that 75 to 80% of all photosystem II centers are localized in grana partition regions.

The data presented support the concept that the intramembranous particles of the EF face visualized on freeze-fractured chloroplast lamellae represent a central photosystem II reaction center complex plus associated light-harvesting chlorophyll protein. The fact that the EF particles of stroma lamellae are smaller than those of grana regions can be directly correlated to the presence of photosystem II units with small antennae chlorophyll assemblies in stroma lamellae.

There have been consistent reports in the literature which suggest that almost all of the photosystem II of normal, fully developed chloroplasts is localized in the grana membranes. When chloroplasts are fractionated by mechanical means, the isolated stroma lamellae have low photosystem II activity, whereas the grana membrane fractions have high photosystem II activity (6, 11, 19). In addition, the cytochemical staining studies of Weier *et al.* (22) demonstrated that the photoreduction of TNBT[3] (a photosystem II-dependent reaction) is localized in the partition regions of the chloroplast membranes.

[3] Abbreviations: DCIP: 2,6-dichloroindophenol; DPC: diphenyl carbazide; EF: exoplasmic fracture face (inner half-membrane leaflet); $EF_s$: inner membrane leaflet, appressed (stacked) membrane regions; $EF_u$: inner membrane leaflet, unappressed (unstacked) membrane regions; PF: protoplasmic fracture face (outer half-membrane leaflet); $PF_s$: outer membrane leaflet, appressed membrane regions; $PF_u$: outer membrane leaflet, unappressed membrane regions; TNBT: tetranitro-blue tetrazolium.

The concept that all photosystem II may be restricted to the grana has been challenged by Hall and co-workers (9, 10). Using ferricyanide as a Hill electron acceptor, and precipitating the reduced ferrous compound as an insoluble cupric salt visible by electron microscopy, they presented evidence for localization of photosystem II in both stroma and grana lamellae. Similar results have been obtained by Nir and Pease (15).

Related to the question of where the photochemically reactive complexes are localized along the lamellae is the problem of identifying the structural organization of photosystem II complexes within the membrane. Arntzen *et al.* (5) used the freeze-fracture procedure for electron microscopy to examine digitonin-derived, purified photosystem I preparations and photosystem II-enriched submembrane fragments. The photosystem I preparations were found to contain particles that were morphologically identical to those of the PF face of intact membranes, whereas the photosystem II-enriched preparation was correspondingly enriched in particles morphologically identical to those of the EF face of intact membranes. (Current terminology denotes the outer half-membrane leaflet exposed in freeze-fractured chloroplasts as the PF face. The inner half-membrane leaflet exposed by the fracture is the EF face [8].) Arntzen *et al.* (5) proposed that the different freeze-fracture particles were markers of sites of localization of the different photosystems.

It was first observed by Sane *et al.* (19) that there is an unequal distribution of particles along the EF freeze-fracture face of chloroplast lamellae. The density of the EF particles on stroma lamellae was found to be considerably lower than the density within partitions (appressed membrane regions) of grana stacks. This has been verified by Ojakian and Satir (16) and by Staehelin (20). Although the $EF_u$ particles of the stroma membranes are smaller in size than the $EF_s$ particles observed in grana regions, Staehelin concluded that all of the EF particles are related to the same basic membrane component. Arntzen *et al.* (3), in a recent structural study of greening chloroplast membranes, demonstrated that addition of the light-harvesting pigment protein to photosystem II during membrane differentiation resulted in an increase in size of the EF particles. They therefore proposed that the EF particles consist of a photosystem II "core" complex surrounded by varying amounts of light-harvesting pigment protein. It was suggested that the existence of various size classes of EF particles can be explained by the presence of varying amounts of the light-harvesting complex aggregated upon the core photosystem II unit.

From the structural studies of Arntzen *et al.* (3), certain predictions can be made concerning the partitioning and characterization of photosystem II activity along the chloroplast membrane. First, the amount of photosystem II activity to be found in the stroma membranes should be directly correlated with the numbers of EF particles localized in those membrane regions. Second, in membrane regions containing small size classes of EF freeze-fracture particles, the photosystem II units should have a

smaller photosynthetic unit size. These predictions will be shown to be correct in the current study.

## MATERIALS AND METHODS

Chloroplasts were isolated from 2-week-old pea seedlings (*Pisum sativum* L., Morse's Progress No. 9) grown in vermiculite moistened with half-strength Hoagland solution.[4] Illumination was provided by fluorescent light banks (16-hr photoperiod).

**Freeze-fracture Specimen Preparation.** Plastids for freeze-fracture studies were isolated by grinding the pea leaves in a Waring Blendor in a solution containing 0.4 M sorbitol, 0.1 M Na-Tricine (pH 7.8), and 5 mM $MgCl_2$. The brei was filtered through four, then 12 layers of cheesecloth and centrifuged at 1000$g$ for 10 min. The pellet was resuspended in the same solution as above containing 35% glycerol and centrifuged again at 5000$g$. Replicas of pellet samples were made on a Balzer's BA 360 and examined with a Phillips EM300.

**Fractionation of Chloroplast Membranes.** Fractionation studies were performed on pea chloroplasts isolated in a grinding solution of 0.4 M sorbitol, 0.1 M Na-Tricine (pH 7.8), and 5 mM $MgCl_2$. After a 10-min centrifugation at 1000$g$, the pellet was resuspended to 0.5 mg Chl/ml in 0.2 M sorbitol, 1 mM Na-Tricine (pH 7.8), 2 mM NaCl, and 5 mM $MgCl_2$. This plastid suspension was passed once through a French pressure cell and centrifuged at 40,000$g$ for 30 min. The supernatant solutions were retained as the stroma membrane fraction; the pellets (grana-enriched preparations; 6, 17, 19) were resuspended in the above mentioned sorbitol-Tricine-NaCl-$MgCl_2$ solution. Chlorophyll concentrations and Chl $a/b$ ratios were determined according to the method of Arnon (2).

**Iodination Experiments.** For studies of membrane iodination, pea leaves were ground in either one of two different solutions. The first contained 0.4 M sorbitol, 0.1 M Na-Tricine (pH 7.8), and 175 mM NaCl; the second solution was identical except for the omission of NaCl. After a centrifugation at 1000$g$ for 10 min, the pellets were rapidly dispersed in a solution containing 0.15 M sorbitol and 2 mM Na-Tricine (pH 7.8). Following this brief treatment, which resulted in the rupture of any intact chloroplasts, sufficient NaCl for a final concentration of 200 mM or sufficient EDTA (pH 7.8) for a final concentration of 5 mM was then added to the samples derived from grinding in salt concentrations of 175 and 0 mM NaCl, respectively. The suspensions were centrifuged at 5000$g$ for 10 min, washed once in identical salt or EDTA solutions, and the resuspended in 0.15 M sorbitol and 2 mM Na-Tricine (pH 7.8) (containing either 200 mM NaCl or 5 mM EDTA) to a final Chl concentration of 200 $\mu$g/ml. The preparations at this point consisted of washed lamellae with little or no soluble, stroma-derived protein remaining.

Sufficient glutaraldehyde for a final concentration of 0.5% was added to each of the washed lamellae samples described above. The chloroplasts were stirred in the fixative at 5 C for 5 min, then centrifuged at 5000$g$ for 10 min. The pellets were washed once with the appropriate resuspension solution to remove glutaraldehyde and centrifuged again at 5000$g$ for 10 min. The pellets were resuspended in a small volume of resuspension solution used in the last washing step above.

Iodination reactions were conducted in a 2-ml volume of the respective resuspension solution containing 200 $\mu$g Chl, 0.1 mg lactoperoxidase (Sigma Chemical Co.), 0.4 mM KI, and varying amounts of $H_2O_2$. Reactions were initiated by the addition of lactoperoxidase (as previously described [7]), and were then allowed to incubate for 2 min at 23 C.

For thin section electron microscopy, aliquots of the glutaraldehyde-fixed chloroplast preparations were washed five times with 0.05 M phosphate buffer (pH 7) and were then postfixed with 1% $OsO_4$. Chloroplast pellets were dehydrated in a graded acetone series and were embedded in Epon. The blocks were sectioned with a diamond knife. Sections were stained in a uranyl acetate-lead citrate staining series and examined with an RCA EMU-4 electron microscope.

## RESULTS

**Freeze-fracture Analysis of Stroma Lamellae and Grana.** Figure 1 is an electron micrograph of pea chloroplast membranes prepared by the freeze-fracture technique. An analysis of the density of the intramembranous particles revealed that the particle density in the unstacked ($EF_u$) membranes is only 33% of that of stacked ($EF_s$) membrane regions whereas numbers of particles in $PF_u$ *versus* $PF_s$ regions are equal (Table I). Measurements of the lengths of unstacked *versus* stacked chloroplast membranes in thin sectioned plastid preparations of similar material demonstrated that approximately 38% of all chloroplast membranes are unstacked. Considering both area and densities, we can calculate that the $EF_u$ particles account for 17% of the total number of EF particles in our chloroplast preparations. (Calculations: 38% unstacked membranes/plastid $\times$ 487 particles/unit area of unstacked membranes = 185; 62% stacked membranes/plastid $\times$ 1,472 particles per unit area of stacked membranes = 913. On a total unit area basis, there are therefore 1,098 [185 + 913] EF particles. The percentage of EF particles in unstacked membranes is 185/1,098 = 17%.)

Measurements of particle sizes on the $EF_u$ and $EF_s$ faces were conducted. The particles of the $EF_s$ face ranged in diameter from 50 to 195 Å with most particles being in two size classes of about 110 and 165 Å. The mean particle diameter of the $EF_s$ particles was 128 Å. Particles of the $EF_u$ face ranged in size from 50 to 155 Å. The mean particle diameter of the $EF_u$ particles was 95 Å.

**Characterization of Photosystem II Activities in Membranes Derived from Grana and Stroma Lamellae.** The various membrane fractions were characterized after French pressure cell treatment of chloroplast lamellae at various pressures (Table II). It is apparent from these data that all pressure cell treatments have a detrimental effect upon photosystem II activity since the rates of DCIP reduction in all membrane fragments were somewhat lower than rates for untreated lamellae. This was an apparently irreversible inhibition, since activity was not restorable by the addition of an exogenous donor to photosystem II (diphenyl carbazide). The light fraction obtained after differential centrifugation of the French pressure cell-treated chloroplasts has been previously shown to consist of membrane fragments derived from stroma lamellae (17, 19). It should be noted that increasing amounts of this membrane fraction were recovered as the pressure was increased during the French pressure cell treatment. Previous studies demonstrated that multiple sequential pressure treatments are necessary for maximum release of stroma lamellae (6). Since the 1,000 p.s.i. treatment yielded membrane fragments with the highest photochemical activity, this pressure was chosen for membrane preparation in subsequent analyses.

The effect of various exciting light intensities on photosystem II activity (DCIP reduction with $H_2O$ as the electron donor) in grana and stroma lamellae was examined (Fig. 2A). The photosystem II activity of the grana membranes began to saturate at an incident light intensity of 4 $\times$ $10^5$ ergs $cm^{-2}$ $sec^{-1}$, whereas the stroma lamellae photosystem II activity did not saturate under the light intensities used in this experiment. Photosystem I activity for these two samples (Fig. 2B), in contrast, saturated at approximately the same light intensities. This indicates that the differences in intensity dependence of photosystem II activity in

FIG. 1. Fracture faces of chloroplast membranes isolated from mature pea leaves. Four distinct fracture faces are distinguishable in such a preparation: $PF_u$, $PF_s$, $EF_u$, and $EF_s$ (current nomenclature as defined by ref. 8). × 125,000.

the grana and stroma lamellae preparations were related to different antennae sizes of the photosystem II complexes in the two different samples, and not due to general membrane damage in the preparation.

**Localization of Photosystem II by Lactoperoxidase-catalyzed Iodination.** We have previously found that lactoperoxidase-cata-

lyzed iodination of chloroplast lamellae inactivates photosystem II reaction centers (7). This reaction requires interaction between the relatively large lactoperoxidase enzyme and its substrate (tyrosine residues) on the membrane surface. In this study, membrane analysis by iodination was used upon chloroplasts in either a stacked or unstacked configuration. Preliminary

Table I. Analysis of Particle Densities on the
Fracture Faces of Pea Chloroplast Membranes

| Fracture Face | Particle Density No. of particles/micron$^2$ |
|---|---|
| $PF_s$ | 4496 ± 401 |
| $PF_u$ | 4416 ± 385 |
| $EF_s$ | 1472 ± 132 |
| $EF_u$ | 487 ± 115 |

Table II. Effect of Various Pressures During French Pressure
Cell Treatment on Fractionation Characteristics

Chloroplasts were given a single treatment with a French pressure cell. The grana and stroma lamellae were separated by centrifugation at 40,000g for 30 min (grana membranes were pelleted; stroma lamellae remained in the supernatant). Chlorophyll concentrations, Chl a/b ratios, and rates of photosystem II mediated electron transport (H₂O → DCIP and DPC → DCIP) were determined for the two fractions. Reaction mixtures contained in a volume of 2 ml: 50 mM Na phosphate (pH 6.7), 0.04 mM DCIP, 1.25 mM DPC, 5 mM NH₄Cl, and 50 μg chlorophyll. DPC was added to the reaction mixtures as a photosystem II electron donor (21).

| Pressure lbs/in$^2$ | Fraction | % Recovery of Chl | Chl a/b Ratio | Photosystem II Activity H2O → DCIP μmole DCIP reduced·mg Chl$^{-1}$·hr$^{-1}$ | DPC → DCIP |
|---|---|---|---|---|---|
| 0 | grana | > 99 | 3.01 | 187 | 187 |
|  | stroma | < 1 |  |  |  |
| 1000 | grana | 98.5 | 2.86 | 142 | 147 |
|  | stroma | 1.5 | 5.43 | 26 | 26 |
| 2000 | grana | 95.8 | 2.87 | 107 | 108 |
|  | stroma | 4.2 | 5.43 | 15 | 16 |
| 4000 | grana | 92.6 | 2.79 | 93 | 98 |
|  | stroma | 7.4 | 5.66 | 19 | 18 |

experiments indicated that chloroplasts with the desired membrane configuration could be consistently obtained by conducting all isolation manipulations in solutions of constant cationic concentrations. The work of Murakami and Packer (14) has shown that resuspending isolated chloroplasts in 200 mM NaCl results in tightly stacked grana. This is verified in Figure 3A. We have found that by preparing chloroplasts in 5 mM EDTA (pH 7.8), the plastid membranes are unstacked (Fig. 3B). It should be noted that treating the membranes with 5 mM EDTA does not remove the coupling factor (thereby exposing new iodination sites).

Our earlier work with lactoperoxidase-catalyzed iodination (7) showed that membrane damage occurred at high levels of iodination (high H₂O₂ concentrations). Such damage could be prevented by stabilizing the chloroplast lamellae with glutaraldehyde prior to iodination. This procedure was adopted in the current study.

When the stacked chloroplast lamellae (suspended in 200 mM NaCl; Fig. 3A) were iodinated over a range of substrate (H₂O₂) concentrations, there was only a slight inhibition of photosystem II activity (Fig. 4). In contrast, in unstacked chloroplasts, photosystem II activity was rapidly and nearly totally inhibited by iodination. Since these samples were fixed with glutaraldehyde prior to iodination, all effects are due to direct action of the lactoperoxidase iodination on photosystem II centers (see ref. 7).

Although it seemed likely that the configuration of the chloroplast membranes was the primary determinant regulating the extent of iodination inactivation of the photosystem II centers, it was necessary to rule out the possibility that the limited action of the enzyme in "high salt chloroplasts" might simply be due to direct effects of salt on the lactoperoxidase enzyme. For this reason, iodination of free tyrosine was carried out under experimental conditions identical to those used for the iodination of chloroplast membranes. Free tyrosine was incubated with lactoperoxidase at various H₂O₂ concentrations for 2 min; and the change in $A$ at 290 nm measured after that time. Morrison and
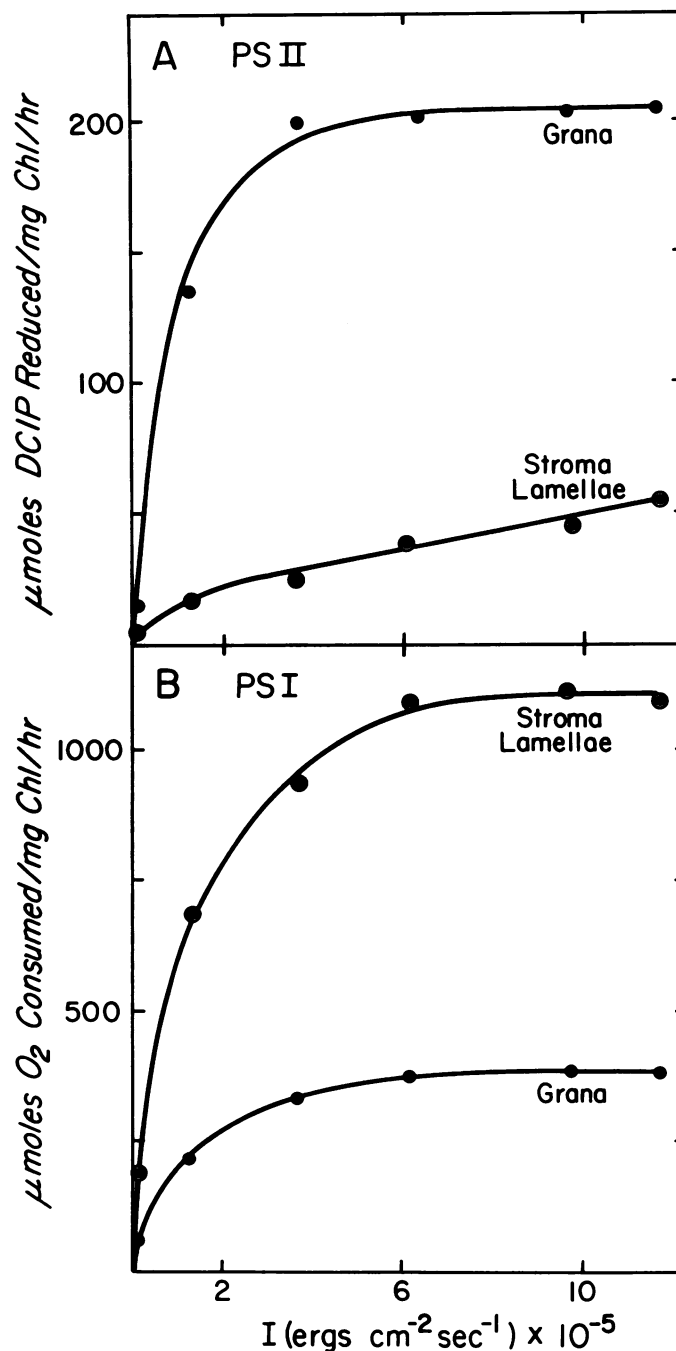


FIG. 2. Effects of light intensity on photosystem II activity (A) and photosystem I activity (B) for French pressure cell-derived grana and stroma membrane fractions. Reaction conditions for photosystem II activity measurements were as described in Table II with the exception that no DPC was added to the reaction mixture. The reaction mixture for photosystem I activity measurements contained, in a volume of 1.5 ml: 1 mM methyl viologen, 1 mM NaN₃, 5 mM NH₄Cl, 0.5 mM DCIP, 5 mM Na-ascorbate, 3 × 10⁻⁵ M DCMU, 5 mM MgCl₂, 10 mM NaCl, 1 mM Na-Tricine, 0.15 M sorbitol, and 10 μg Chl. Autooxidation of the methyl viologen was monitored with a Clark electrode. Illumination was with red light obtained through Corning 2-58 and 1-69 filters. Light intensities were measured with a Yellow Springs/Kettering model 65 radiometer.

Bayse (13) have previously demonstrated that iodination of tyrosine results in a change of the $A$ of the amino acid at 290 nm. There was little effect of salt concentration on the activity of the lactoperoxidase enzyme (Fig. 5).
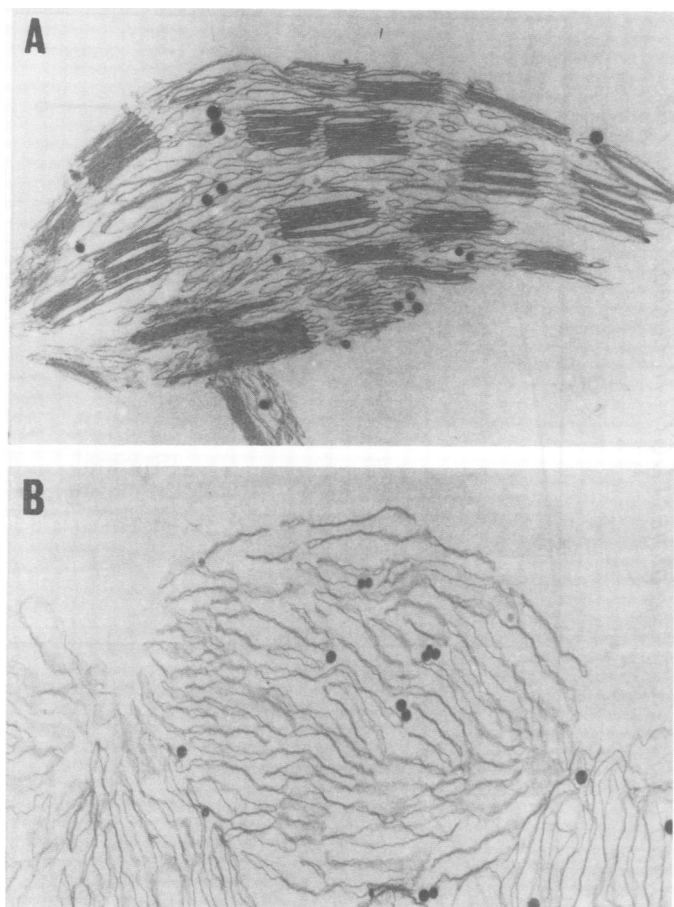
FIG. 3. Chloroplasts prepared in the presence of 200 mM NaCl (A) or 5 mM EDTA (B).
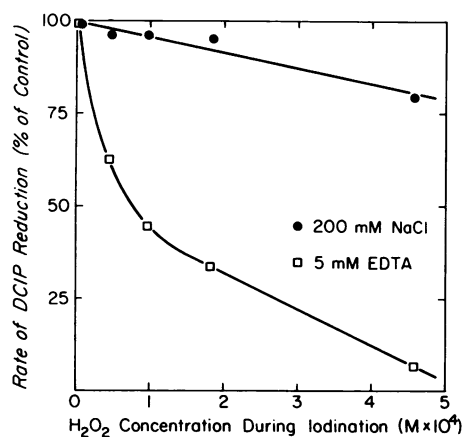


FIG. 5. Iodination of free tyrosine in 200 mM NaCl (O) or 5 mM EDTA (□). Reaction mixtures for iodination contained $5 \times 10^{-4}$ M tyrosine. The reactions were initiated by the addition of $H_2O_2$ to give the. final concentration indicated. The reaction mixture $A$ at 290 nm was determined before and after a 2-min incubation with $H_2O_2$.

membrane regions. In addition, the particles within these grana stacks are, on the average, considerably larger than particles in unstacked membranes. These data are in agreement with earlier structural studies of fully differentiated membranes (3, 16, 20). There is a nonuniform distribution of photosystem II activity along plastid lamellae; grana membranes are enriched in photosystem II activity while stroma lamellae show high photosystem I but low photosystem II rates (Table II, references 4, 6, 11, 17). Correlation of structure and function suggests that the photosystem II activity is not related to particles of the PF face since particle numbers on the $PF_s$ and $PF_u$ faces are constant while photosystem II activity of the unstacked membranes is markedly reduced. The reduced content of photosystem II centers in stroma lamellae does correlate to reduced numbers of $EF_u$ particles, however. In the following discussion, we will emphasize the quantitative correlation between these two parameters. The smaller average size of $EF_u$ particles as compared to $EF_s$ particles will also be related to a smaller light-harvesting pigment protein complement of photosystem II centers in stroma lamellae.



FIG. 4. Effect of iodination on photosystem II activity of chloroplasts having stacked membranes (200 mM NaCl) or unstacked membranes (5 mM EDTA). Photosystem II photochemical activity was determined by measuring DCIP photoreduction in a dual beam spectrophotometer modified for sample illumination. The rates presented are activities which were inhibited by $1 \times 10^{-5}$ M DCMU. Control rates for the 200 mM NaCl and 5 mM EDTA samples were 52 and 88 $\mu$mol DCIP reduced/mg Chl/hr, respectively.

## DISCUSSION

This study has shown that there is a nonuniform pattern of distribution of intramembranous particles along the EF face of chloroplast lamellae examined by freeze-fracture techniques (Fig. 1, Table I). Most particles are localized within the fused
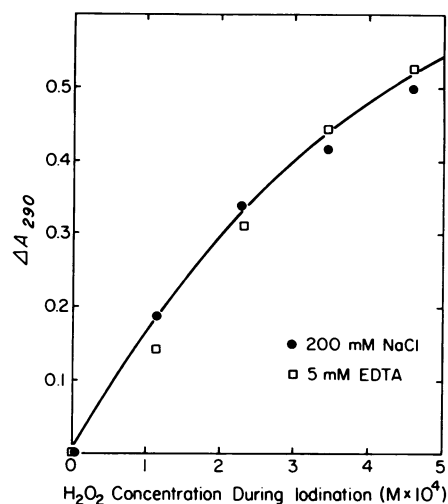
**Association of the Light-harvesting Chlorophyll a/b Protein with Particles of the EF Fracture Face.** In a previous study of developing chloroplast membranes, we established that incorporation of the light-harvesting Chl $a/b$ protein into differentiating membranes corresponds to an increase in size of the particles of the EF fracture face (3). From these data, a model for the chloroplast membrane (Fig. 6) was constructed that depicts protein complexes embedded within a lipid bilayer matrix (3). Particles which cleave with the EF fracture face were suggested to contain an 80 Å "core" unit which is the photosystem II reaction center complex. From 0 to 4 aggregates of light-harvesting Chl $a/b$ protein were thought to be associated with this core unit, thus forming EF particles of various sizes. It has been previously shown (3, 16, 20) that the $EF_u$ particles (located on the unstacked stroma lamellae) of the chloroplast membranes are of a smaller average size than the $EF_s$ particles (located in stacked membrane regions). If the model of Figure 6 is correct, then the $EF_u$ particles should correspond to photosystem II units with a smaller average antenna Chl complement than $EF_s$ particles. This correlation is supported by the data of Figure 2. Photosystem II activity of stroma lamellae did not show light saturation whereas grana membrane preparations saturated at moderate light intensities. The stroma lamellae also exhibited lower photosystem II rates than the grana membranes at all light
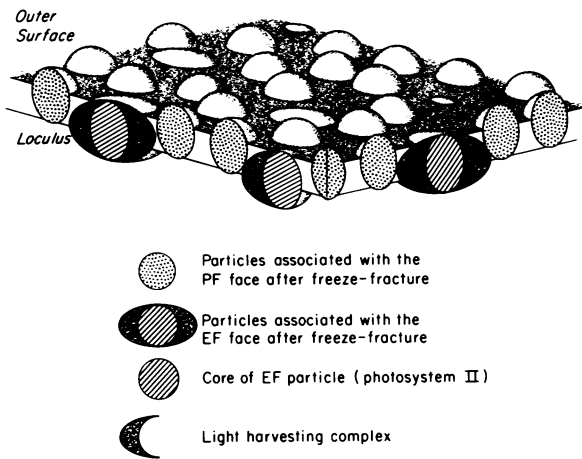
FIG. 6. Model illustrating the spatial relationships for the particulate subunits of the chloroplast membrane. Protein complexes are thought to be embedded in a lipid matrix. Freeze-fracturing results in the splitting of the lipid bilayer exposing intramembranous particles of the PF (outer leaflet) and EF (inner leaflet) fracture faces. The PF particles are predominantly of a single size class of 70 to 77 Å. The EF particles are of two size classes, 105 and 164 Å. Variations in the size of the EF particles are considered to be due to different amounts of the light-harvesting Chl a/b protein associated with the 80 Å photosystem II core complex. While grana membranes contain both the 105 and 164 Å diameter particles, stroma lamellae contain only the 105 Å particles plus some 80 Å core particles with no associated light-harvesting Chl a/b protein.

intensities. These observations can mean either ($a$) that the stroma lamellae contain fewer photosystem II units, each with a smaller photosynthetic unit size than the grana photosystem II units; or ($b$) that the stroma lamellae contain normal numbers of photosystem II units, but that these units are inefficient in their function. The second possibility is unlikely, since numerous previous studies (see refs. 4 and 17) have found a reduction in photosystem II-associated constituents (such as manganese, C550, and Cyt $b_{559}$) in stroma lamellae. The first alternative, that is, small photosystem II units, is therefore consistent with the data presented, and with the model of Figure 6. We conclude that the smaller sized $EF_u$ particles of stroma lamellae correspond to photosystem II units with little associated light-harvesting Chl a/b protein.

**Quantitative Evaluation of the Distribution of Photosystem II between Stroma Lamellae and Grana Partition Regions.** We have found that 17% of the EF particles are localized in unstacked membrane regions. According to the model of Figure 6, therefore, a similar amount of photosystem II complexes should be found in the stroma lamellae if the EF particles are indeed a structural equivalent to photosystem II centers. We have found that a quantitative evaluation of the photosystem II activity cannot be reliably obtained from mechanical membrane fractionation procedures since the fragmentation process results in some inactivation of the photochemical complexes (Table II). The high pressures needed in the French press to obtain pure grana membrane preparations result in severe reductions of photosystem II activity. We have therefore utilized another specific photosystem II identification technique that can be used with intact membrane preparations: lactoperoxidase-catalyzed iodination.

Lactoperoxidase-catalyzed iodination is a highly specific means of inactivating surface-exposed photosystem II in chloroplast membranes (7). Phillips and Morrison (18) showed that the lactoperoxidase enzyme, due to its large mol wt (75,000), is unable to penetrate membranes and can therefore react only with proteins at the surface of a membrane. We will also assume that this large enzyme would also be unable to penetrate between the tightly appressed thylakoids of stacked grana mem-

branes. Consequently, lactoperoxidase may only iodinate proteins of photosystem II which are freely accessible to the enzyme, i.e. not in the partition regions of the grana stacks in normal, stacked chloroplast lamellae preparations.

Our results indicated that in fully stacked chloroplasts, about 20 to 25% of photosystem II activity is lost during iodination (Fig. 5). This can be interpreted to mean that 75 to 80% of photosystem II is localized in the partition regions of the grana, but that a significant percentage (20-25%) is localized in regions of the membrane accessible to the lactoperoxidase enzyme. In other related studies, Koenig et al. (12) have also estimated that a minimum of 20% of the photosystem II of chloroplast membranes is not localized in the partitions of grana stacks, based on immunochemical studies. There is a close correlation between the measured number of photochemically active photosystem II complexes in stroma lamellae and the number of $EF_u$ particles present when data are presented as a percentage of the totals observed in the whole plastid.

**Evaluation of Chloroplast Membrane Differentiation.** The data discussed above have clearly supported the concept that the intramembranous particles observed on the EF face of freeze-fractured chloroplast membranes are the morphological equivalent of a photosystem II complex and its associated antenna pigment proteins. The fact that the $EF_u$ particles are smaller (contain less light-harvesting pigment protein) is consistent with a hypothesis put forward by Arntzen and Briantais (4) in which it was suggested that stroma lamellae represent incompletely differentiated regions of chloroplast membranes. Membrane development studies (1, 3) have shown that grana formation and appearance of the light-harvesting Chl a/b protein in the chloroplast membrane are directly correlated during the final stages of membrane differentiation, and that the aggregation of the EF particles into stacked membrane regions corresponded to the time of addition of the light-harvesting pigment proteins to the photosystem II unit.

The importance and functional reason for aggregation of the fully differentiated photosystem II units into regions of grana stacking are yet to be determined.

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